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(54) Title: IMPROVED β,β,-DIHYDROXY MESO-SUBSTITUTED CHLORINS, ISOBACTERIOCHLORINS, AND BACTERIOCHLORINS

(57) Abstract

Improved β,β'-dihydroxy meso-substituted chlorin, bacteriochlorin or isobacteriochlorin compounds are provided as photosensitizers. Pharmaceutical compositions and photodynamic therapy comprising them are also disclosed.

IMPROVED β,β'-DIHYDROXY MESO-SUBSTITUTED CHLORINS, ISOBACTERIOCHLORINS, AND BACTERIOCHLORINS

RELATED APPLICATIONS

This application claims benefit of priority from U.S. Provisional Application 60/129,324, filed April 14, 1999, which is hereby incorporated by reference as if fully set forth.

Field of the Invention

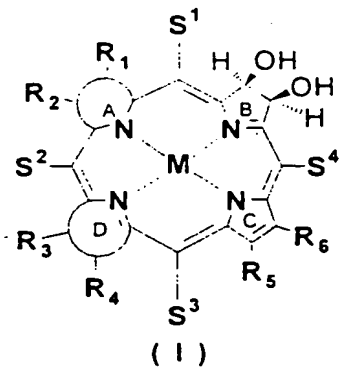
The present invention relates to certain improved dihydroxy chlorin, bacteriochlorin or isobacteriochlorin compounds and their preparation for use in photodynamic therapy (PDT). In particular, the invention relates to analogs of dihydroxylated β,β'-unsubstituted tetrapyrrolic macrocycles that have increased toxicities. Many of these compounds are useful photosensitizers in PDT for mediating the destruction of unwanted cells or tissues or other undesirable materials by irradiation.

Background Art

Photodynamic therapy (PDT) generally involves the administration of compounds that are capable of absorbing light, typically in the visible range, but also in the near ultraviolet, followed by irradiation of locations in the subject for which a toxic, inhibitory or modulatory effect is desired. PDT was initially developed using hematoporphyrin and related compounds in the treatment of tumors, as it appeared that these compounds would "home" to locations containing rapidly dividing cells. The tumor could then be irradiated with light absorbed by the hematoporphyrin and destruction of the surrounding tissue resulted (for example, see US Patent Nos. 4,932,934 and 5,283,255). PDT has since been shown to be useful for treatment of my other conditions, including ocular diseases characterized by unwanted neovascularization, such as age-related macular degeneration (see US patent Nos. 5,756,541 and 5,798,349), the inhibition of secondary cataract formation in the eye (US Patent No. 6,043,237), the impairment of blood-borne targets such as leukemic cells and immunoreactive cells (US Patent Nos., 5,776,966, 5,807,881 and 5,868,695) the removal of unwanted microorganisms (US Patent No. 5,360,734), the removal of

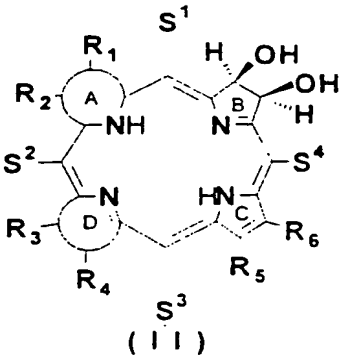
aqueous phase and therefore many photosensitizer molecules can bind to each lipoprotein. The relative binding of tetrapyrroles to lipoproteins has been shown to increase with decreasing polarity (Bonnett, R. SPIE 1993, 2078, 74). The partitioning of hydrophobic photosensitizers is significant as these dyes tend to aggregate in aqueous systems. The extent of aggregation is dependent upon the polarity of the substituents on the porphyrin skeleton (Redmond, R.W.; Land, E.J.; Truscott, T.G. in Advances in Experimental Medicine and Biology. Volume 193. Kessel, D. Ed.; Plenum, New York. 1985, 293). Only monomeric nonaggregated molecules are photoactive and therefore any aggregation will decrease the observed cytotoxicity of the drug (Ibid, p. 301).

Hydrophobic photosensitizers must, therefore, be properly formulated in order to counteract their natural tendency to aggregate in aqueous systems. An advantage of hydrophobic drugs is their preferential binding to lipoproteins as tumor cells express a much larger number of receptors for low density lipoproteins (LDL) than do most normal cells (Spikes, J.D. in Light in Biology and Medicine Vol. 1. Douglas, R.A.; Moan, J.; Dall'Acqua F. Eds.; Plenum, New York, 1988, p. 105). These receptors specifically recognize LDL and promote their internalization by cells via the formation of coated pits. Photosensitizers that bind to LDL are endocytosed by the neoplastic cells along with the lipoprotein (Fisher, A.M.R.; Murphree, A.L.; Gomer, C.J. Lasers in Surgery and Medicine 1993, 17, 2). Once inside the cell, the photosensitizer is released into the cytoplasm and binds to apolar endocellular matrices such as mitochondria, lysosomes and plasma membranes. A photosensitizer will be most effective if it displays an affinity for tumor cells versus normal cells because low cytotoxicity of such a drug can be overcome by increasing the dose. In the early 1980s, ortho-, meta- and para-isomers of meso-tetra(hydroxyphenyl)porphyrin were investigated for use as photosensitizers (Berenbaum, M.C.; Akande, S.L.; Bonnett, R.; Kaur, H.; Ioannou, S.; White, R.D.; Winfield, U.-J. Br. J. Cancer 1986, 54, 717). In order to increase the absorption in the red region, the analogous chlorins and the meta-hydroxy substituted bacteriochlorin were synthesized (Bonnett, R.; Berenbaum, M. in Photosensitizing Compounds: their Chemistry, Biology and Clinical Use. Wiley, Chichester. Ciba Foundation



(I)

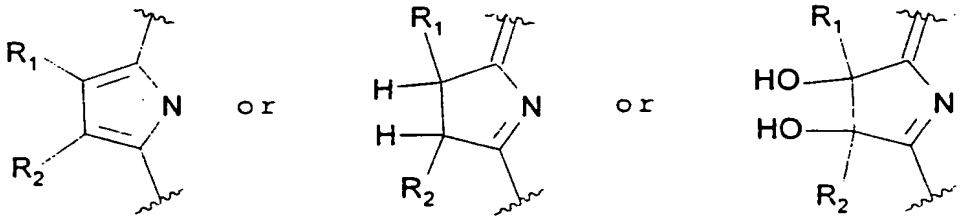
or



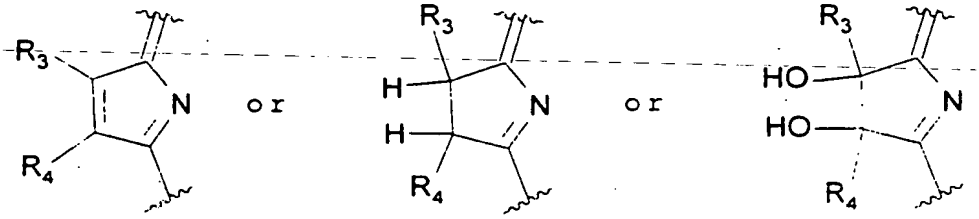
(II)

wherein M is a metal selected from the group consisting of Ni(II), Cu(II), Zn(II), Fe(III)Cl, Sn, Ge, Si, Ga, Al, Mn(III), Gd(III), In and Tc:

A is a ring having the structure:



D is a ring having the structure:



R_1 through R_6 are independently a hydrogen atom, a lower alkyl group, a lower alkyl carboxylic acid or acid ester group, keto, hydroxy, nitro, amino or a group that, taken together with another ring, ring substituent or meso-substituent, forms a fused 5- or 6-membered ring; and

methylene chloride. Typical chlorin ^{1}H-NMR spectra were obtained for the diol chlorins.

Figure 3 shows the formula of a dihydroxychlorin of the invention, where R_2 through R_6 are independently a hydrogen atom, a lower alkyl group, a lower alkyl carboxylic acid or acid ester group, keto, hydroxy, nitro, amino, bromo, fluoro, or iodo group.

Figure 4 shows the formula of a meso-tetraphenyl-2,3,12,13-tetrahydroxybacteriochlorin of the invention, where R_2 through R_6 are independently a hydrogen atom, a lower alkyl group, a lower alkyl carboxylic acid or acid ester group, keto, hydroxy, nitro, amino, bromo, fluoro, or iodo group.

Figure 5 shows the osmium tetroxide mediated oxidation of tetraphenylporphyrins.

Figure 6 shows the formation of meso-tetraphenyl-2,3-dihydroxy-12,13-dihydrobacteriochlorin (compound in the center) via a reaction of meso-tetraphenylchlorins with 1.1 eq. osmium tetroxide, pyridine, $CHCl_3$ and gaseous H_2S (reaction on the left) or a reaction of 2,3-vic-dihydroxy-meso-tetraphenylchlorin by reflux with pyridine, K_2CO_3, and p-toluenesulfonylhydrazine (reaction on the right).

Figure 7 shows the formation of two isomers of 2,3,12,13-bis-(vic-dihydroxy)bacteriochlorins by dihydroxylation of 2,3-vic-dihydroxy-meso-tetraphenylchlorin with one equivalent of osmium tetroxide.

Figure 8 shows the formation of two isomers of (*meso*-tetraphenyl-2,3,7,8-tetrahydroxyisobacteriochlorinato)zinc(II) from the zinc diol of 2,3-vic-dihydroxy-meso-tetraphenylchlorin by reaction with one equivalent of osmium tetroxide in 2.5% pyridine/$CHCl_3$.

Figure 9 shows the structure of N-methyl tetraphenylporphyrin (compound 31).

Modes of Carrying Out the Invention

The β,β'-dihydroxy meso-substituted chlorin, bacteriochlorin or isobacteriochlorin compounds of the invention have formula (I) or formula (II), as described and shown above. M in formula (I) can be any metal species that is capable of forming the complex of formula (I), but is preferably selected from the group

$-CH_2CH_2CH_2COOCH_2CH_2CH_3$, $-CH_2CH(CH_3)_2COOCH_2CH_3$; keto; hydroxy; nitro; amino; or the like.

Further, R_1 and R_2, R_3 and R_4, or R_5 and R_6, can be taken together with another ring, ring substituent or meso-substituent to form a fused 5- or 6-membered ring. The fused 5- or 6-membered ring so formed may be any saturated or unsaturated, carbocyclic or heterocyclic 5- or 6-membered ring that does not interfere with the osmylation and reduction reaction steps of the invention. Examples of such rings include cyclopentane, furan, thiophene, pyrrole, isopyrrole, 3-isopyrrole pyrazole, 2-isoimidazole, 1,2,3-triazole, 1,2,4-triazole, 1,2-dithiole, 1,3-dithiole, 1,2,3-oxathiole, isoxazole, oxazole, thiazole, isothiazole, 1,2,3-oxadiathiazole, 1,2,4-oxadiazole, 1,2,5-oxadiazole, 1,3,4-oxadiazole, 1,2,3-dioxazole, 1,2,4-dioxazole, 1,2,5-oxathiazole, 1,3-oxathiole, benzene, cyclohexane, 1,2-pyran, 1,4-pyran, 1,2-pyrone, 1,4-pyrone, 1,2-dioxin, 1,3-dioxin (dihydro form), pyridine, pyridazine, pyrimidine, pyrazine, piperazine, 1,3,5-triazine, 1,2,4-triazine, 1,2,4-oxazine, 1,3,2-oxazine, o-isoxazine, 1,2,5-oxathiazine, 1,4-oxazine, p-isoxazine, 1,2,6-oxathiazine, 1,3,5,2-oxadiazine, morpholine, azepine, oxepin, thiepin, 1,2,4-diazepine, and the like. Preferably, when R_1 and R_2, R_3 and R_4, or R_5 and R_6, form a fused, 5- to 6-membered ring, the ring is a 6-membered ring. Most preferably, when R_1 and R_2, R_3 and R_4, or R_5 and R_6, form a ring, it is a 6-membered carbocyclic ring, i.e., a benzene ring.

In a particularly preferred embodiment, R_1 through R_6 are independently hydrogen, methyl, ethyl, or lower alkyl esters, most preferably being hydrogen, methyl or ethyl.

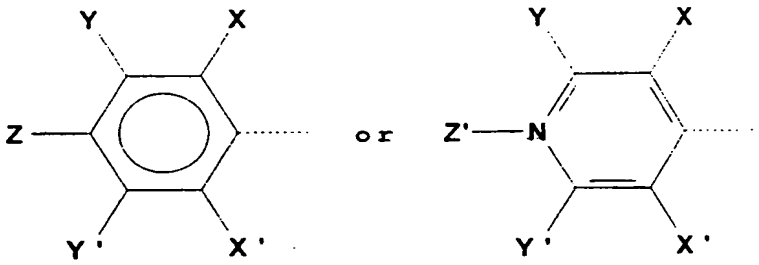
Preferably, at least one of S^1 to S^4 is a phenyl group and the remaining S positions are independently selected from H, any one of a large number of substituted or unsubstituted alkyl groups, substituted or unsubstituted cycloalkyl groups, and aromatic rings. When one or more of S^1 through S^4 is an alkyl group, they preferably have from about 1 to about 18 carbon atoms, more preferably about 1 to 12 carbon atoms and, even more preferably, about 1-6 carbon atoms. Examples of typical alkyl groups are methyl, ethyl, isopropyl, sec-butyl, tert-butyl, n-pentyl and n-octyl.

When one or more of S^1 through S^4 is an alkyl group, it may be unsubstituted or substituted with any group that does not interfere with the osmylation

1,2-benzopyrone, 1,4-benzopyrone, 2,1-benzopyrone, 2,3-benzopyrone, quinoline, isoquinoline, 1,2-benzodiazine, 1,3-benzodianzine, naphthyridine, pyrido[3,4-b]-pyridine, pyrido[3,2-b]-pyridine, pyrido[4,3-b]-pyridine, 1,3,2-benzoxazine, 1,4,2-benzoxazine, 2,3,1-benzoxazine, 3,1,4-benzoxazine, 1,2-benzisoxazine, 1,4-benzisoxazine, anthracene, phenanthrene, carbazole, xanthene, acridine, purine, steroidal compounds and the like.

In a particularly preferred embodiment, both S^2 and S^4 are phenyl groups.

In another embodiment, at least one of S^1 through S^4 has the structure:



wherein X, Y, Z, X', Y' and Z' can be any one of a large number of substituents and are generally used to "fine tune" the biological activity, the biodistribution, the absorption and clearance characteristics, and the physical properties of the desired product. One way in which this may be done by selecting substituents in such a manner that the compound of formula (I) or (II) is an amphiphilic molecule. By "amphiphilic" is meant the molecule becomes more asymmetric, such as

(1) having both (a) a highly polar, water-soluble region and (b) a highly hydrophobic, water-insoluble region; or

(2) having both (a) a nonionic region and (b) an ionic region.

However, it should be noted that the invention also includes β,β'-dihydroxy meso-substituted chlorin, bacteriochlorin or isobacteriochlorin compounds having substantially or exactly identical aryl substituents. Further, any aryl substituent chosen should also have no adverse effect on the ability of the compound to undergo the step "a." and step "b." reactions used to prepare the compounds of the invention.

(5) O-acyl derivatives such as penta-O-acetyl-I-glucose; (6) O-methyl derivatives such as methyl I-glucoside, methyl J-glucoside, methyl I-glucopyranoside, and methyl-2,3,4,6-tetra-O-methyl-glucopyranoside; (7) phenylosazones such as glucose phenylosazone; (8) sugar alcohols such as sorbitol, mannitol, glycerol, and myo-inositol; (9) sugar acids such as gluconic acid, glucaric acid and glucuronic acid, L-gluconolactone, L-glucuronolactone, ascorbic acid, and dehydroascorbic acid; (10) phosphoric acid esters such as I-glucose 1-phosphoric acid, I-glucose 6-phosphoric acid, I-fructose 1,6-diphosphoric acid, and I-fructose 6-phosphoric acid; (11) deoxy sugars such as 2-deoxy-ribose, rhamnose (deoxy-mannose), and fucose (6-deoxy-galactose); (12) amino sugars such as glucosamine and galactosamine; muramic acid and neuraminic acid; (13) disaccharides such as maltose, sucrose and trehalose; (14) trisaccharides such as raffinose (fructose, glucose, galactose) and melezitose (glucose, fructose, glucose); (15) polysaccharides (glycans) such as glucans and mannans; and (16) storage polysaccharides such as I-amylose, amylopectin, dextrins, and dextrans.

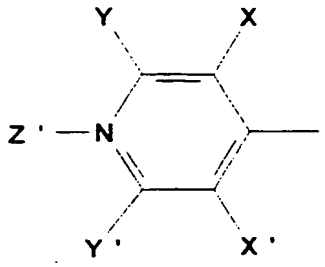
Amino acid derivatives are also useful biologically active substituents, such as those derived from valine, leucine, isoleucine, threonine, methionine, phenylalanine, tryptophan, alanine, arginine, aspartic acid, cystine, cysteine, glutamic acid, glycine, histidine, proline, serine, tyrosine, asparagine and glutamine. Also useful are peptides, particularly those known to have affinity for specific receptors, for example, oxytocin, vasopressin, bradykinin, LHRH, thrombin and the like.

Another useful group of biologically active substituents are those derived from nucleosides, for example, ribonucleosides such as adenosine, guanosine, cytidine, and uridine; and 2'-deoxyribonucleosides, such as 2'-deoxyadenosine, 2'-deoxyguanosine, 2'-deoxycytidine, and 2'-deoxythymidine.

Another category of biologically active groups that is particularly useful is any ligand that is specific for a particular biological receptor. The term "ligand specific for a receptor" refers to a moiety that binds a receptor at cell surfaces, and thus contains contours and charge patterns that are complementary to those of the biological receptor. The ligand is not the receptor itself, but a substance complementary to it. It is well understood that a wide variety of cell types have specific receptors designed to bind hormones, growth factors, or neurotransmitters. However, while these

iodo, bromo, -C(O)-OCH_3, cyano, nitro, or a ligand specific for a biological receptor. In a further preferred embodiment, X, X', Y and Y' and Z is selected from the group consisting of hydrogen, halogen, lower alkyl, lower alkoxy, hydroxy, carboxylic acid or acid salt, carboxylic acid ester, sulfonic acid ester, sulfonic acid or acid salt, nitro, amino, cyano, and a biologically active group. In still another preferred embodiment, at least one of X, Y, Z, X' and Y' is a biologically active group or a substituent that increases the amphiphilic nature of the molecule.

Particularly preferred specific examples of groups that can serve as one or more of S^1 through S^4 include the following:



X	X'	Y	Y'	Z'
-H	-H	-H	-H	-H
-H	-H	-H	-H	$-CH_3$
-H	-H	-H	-H	$-C_6H_{12}OH$
-H	-H	-H	-OH	-H
-H	-H	-OH	-H	-H
-H	-H	-H	$-COONHCH_3$	-H
-H	-H	-H	-H	-benzyl
-H	-H	-H	$-C_6H_{12}OH$	$-CH_3$
-H	-H	$-C_6H_{13}$	-H	$-CH_3$

T(m,m'-OCH_3)PC(OH)$_2$	**16**	H	OCH_3	H	OCH_3	H
T(m-OCH_3,p-OH)PC(OH)$_2$	**17**	H	OCH_3	OH	H	H
T(m,p,m'-OCH_3)PC(OH)$_2$	**18**	H	OCH_3	OCH_3	OCH_3	H
T(o,m,m'-OCH_3)PC(OH)$_2$	**19**	OCH_3	OCH_3	H	OCH_3	H
T(o,p,o'-OCH_3)PC(OH)$_2$	**20**	OCH_3	H	OCH_3	H	OCH_3
T(p-CH_3)PC(OH)$_2$	**21**	H	H	CH_3	H	H
T(o,p,o'-CH_3)PC(OH)$_2$	**22**	CH_3	H	CH_3	H	CH_3
T(p-SO_3H)PC(OH)$_2$	**23**	H	H	SO_3H	H	H
T(p-t-Bu)PC(OH)$_2$	**24**	H	H	t-Bu	H	H

Additionally, meso-5-(p-bromophenyl)-10,15,20-triphenyl-2,3-dihydroxychlorin (compound 26) (mono p-Br TPC(OH)$_2$), meso-5-(p-hydroxyphenyl)-10,15,20-triphenyl-2,3-dihydroxychlorin (mono p-OH TPC(OH)$_2$) (compound 27) and meso-5-(p-nitrophenyl)-10,15,20-triphenyl-2,3-dihydroxychlorin (mono p-NO_2 TPC(OH)$_2$) (compound 28) were synthesized.

Preferred compounds of the invention include those encompassed by the formula of Figure 4 as well as those shown in Table 2.

Other preferred compounds include bacteriochlorins according to the structure shown in Figure 4 and as set forth in Table 2.

Table 2 Tetraphenyl-2,3,12,13-tetrahydroxybacteriochlorins

Compound	Number	R_2	R_3	R_4	R_5	R_6
H_2TPB(OH)$_4$	**29**	H	H	H	H	H
T(o,p,o'-OCH_3)PB(OH)$_4$	**30**	OCH_3	H	OCH_3	H	OCH_3

The above described compounds were tested in vitro for phototoxicity and dark toxicity in L1210 cells as described in the Example section below. Twenty four compounds which were tested for cytotoxicity are listed in Table 3 below along with BPD-MA.

Compound	Rank	LD50 (μM)	Molecular Weight (g/mol)	LD50 (ng/mL)	Rank
T(o,p,o'-CH_3)PC(OH)$_2$ **(22)**	23	12.3	816	10000	19

While it is standard practice to report LD50 values in terms of ng/mL, the above presentation is made since the compounds of the invention span a wide range of molecular weights. Thus, LD50 values would be more accurate when presented in units of μM. Although overall the differences in order were minimal, certain compounds, such as the trimethoxy substituted compounds (118) and (compound 30), were found to be more cytotoxic than the LD50 values presented in units of ng/mL.

The LD50 values in terms of μM also allowed the comparison of the cytotoxicity of the instant compounds with that of other photosensitizers. BPD-MA is a known photosensitizer with an LD50 value of 19 ng/mL, a molecular mass of 718 g/mol, and an LD50 of 0.026 μM which is 70 times more potent than HpD and 45 times more potent than Photofrin™ in sensitizing tumors (Richter, A.M; Waterfield, E.; Jain, A.K.; Sternberg, E.D.; Dolphin, D.; Levy, J.G. Photochem. Photobiol. 1990, 495). Five of the above compounds are more cytotoxic than BPD-MA based on the observed LD50 values. The most cytotoxic compound, diphenyl diol chlorin (125) is extremely cytotoxic: 10 times more potent than BPD-MA, 450 times more potent than Photofrin™ and 700 times more potent than HpD.

The above compounds were also tested for dark toxicity. Dark toxicity refers to the toxicity of the drug to cells in the absence of light. This toxicity is, therefore, not due to singlet oxygen mediated cellular damage. It is critical that potential photosensitizing drugs have low LD50 values in the dark so that photosensitivity after treatment is minimal, Table 4 shows that all of the compounds tested have acceptably low dark toxicity levels.

Table 4 List of compounds in order of decreasing dark toxicity.

Compound	LD50dark	LD20dark	LD50

The singlet oxygen quantum yield, as exemplified by the halogenated diol chlorin compounds, appears to have only a minor influence on the observed cytotoxicity of our compounds, and therefore the difference in toxicities could be reasoned to primarily reflect the cellular uptake of the drugs. A variety of molecular properties have been proposed to be responsible for cellular uptake such as hydrophobicity, amphiphilicity, self-aggregation and the ability to bind to serum protein. An increase in lipophilicity of a photosensitizer has been found to correlate with an increase in cellular uptake of the drug due to an increase in the degree of binding to LDL (Kongshaug, M. Int. J. Biochem. 1992, 24, 1239).

Based on the above, the degree of hydrophobicity and amphiphilicity appear to be important factors in the cytotoxicity of the compounds. Whereas the porphyrin skeleton is essentially hydrophobic, the incorporation of the diol into the skeleton confers a degree of amphiphilicity to the compounds. The highly cytotoxic diphenyl diol chlorin (125) differs from tetraphenyl diol chlorin (compound 3) in that it has two fewer phenyl groups. Phenyl groups are hydrophobic, and their removal alters the degree of hydrophobicity of the molecule and at the same time increases the amphiphilicity. Additionally, the loss of the phenyl group somewhat streamlines the molecule, perhaps improving its cellular uptake. It may be surmised that the increased toxicity of the 5-(p-nitrophenyl)-10,15,20-triphenyl diol chlorin (compound 28) and the 5-(p-hydroxyphenyl)-10,15,20-triphenyl diol chlorin (compound 27) relative to their tetra-substituted analogs is due to the increased amphiphilicity and polarity that a single hydrophilic substituent would confer.

General reactions to produce the above described compounds have been described in U.S. Patent 5,648,485. Briefly, they may be conducted via oxidation of meso-tetraphenylporphyrin or its metallated complex occurred in a solution of chloroform or methylene chloride with a stoichiometric amount of OsO4 in the presence of pyridine. After stirring at room temperature for 5 days in the dark, reduction of the osmate complex with gaseous H_2S yielded the previously unknown 2,3-vic-dihydroxy-meso-tetraphenylchlorin or its metallated analog in ~50% yield with ~40% starting material recovery (see Figure 5). The resultant meso-substituted vic-

Although the above osmium tetroxide based reactions are very useful and promising, there are two major drawbacks to its use for photosensitizer production on an industrial scale. First and most importantly, the osmium tetroxide reagent which is used on an equimolar scale is expensive ($50/g) and relatively toxic. Any measures to decrease the amount of osmium tetroxide required would greatly improve the chances that this reaction might be used on a large scale. Investigations into solving this disadvantage focused on possible catalytic systems and the recycling of the osmium tetroxide reagent. In order to make this reaction catalytic, the reaction time would need to be decreased. This 3-5 day reaction period is the second drawback of the osmium tetroxide oxidation of porphyrins. Efforts in this area were focused on both the reversible modification of the starting materials and also on the use of other substrates as starting materials.

Increasing the distortion of the porphyrin core is known to electronically activate the β,β' bond(s) of the molecule (Khosopour, R.; Hambright, P. J. Chem. Soc., Chem. Comm. 1972, 13). Additionally, it is known that N-alkylated porphyrins are highly distorted, with the most highly distorted porphyrins having the largest alkyl groups bound to one of the inner nitrogen atoms of the porphyrin core (Hassan, M.G.A.; Jackson, A.H.; Johnson, A.W.; Winter, M. J. Chem. Soc., Perkin I 1977, 98). We synthesized an N-alkylated porphyrin for use as a starting material in the osmium tetroxide oxidation reaction: N-methyl tetraphenylporphyrin (N-methyl TPP, compound 31, see Figure 9). Previous studies had indicated that the osmium tetroxide mediated oxidation of N-methyl TPP appeared to be faster than unsubstituted TPP. On average, N-methyl TPP required just 6-12 hours to afford the analogous diol-chlorin. Other N-alkylated tetraphenylporphyrins can also be prepared and used in the present invention.

The improved β,β'-dihydroxy meso-substituted chlorin, bacteriochlorin and isobacteriochlorin compounds of the invention are useful as photosensitizers used in photodynamic therapy (PDT) and as synthetic intermediates for making related photosensitizers. Specifically, these photosensitizers are useful in sensitizing neoplastic cells or other abnormal tissues to destruction by irradiation with visible

resonance imaging. These are also applications where, due the variability possible with respect to the substitution patterns, significantly improved biodistribution properties may be achieved by using the compounds of the invention.

The photosensitizers made from the compounds of the invention can be formulated into pharmaceutical compositions for administration to the subject or applied to an in vitro target using techniques generally known in the art. Additionally, formulations as liposomal compositions has also been demonstrated (copending U.S. patent application 08/489,850). A summary of such pharmaceutical compositions may be found, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA. The compounds of the invention can be used singly or as components of mixtures.

Generally, for the diagnosis or treatment of solid tumors, the compound of the invention, labeled or unlabeled, is administered systemically, such as by injection. Injection may be intravenous, subcutaneous, intramuscular, or even intraperitoneal. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in a liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, glycerol and the like. Of course, these compositions may also contain minor amounts of nontoxic, auxiliary substances, such as wetting or emulsifying agents, pH buffering agents, and so forth.

Systemic administration can be implemented through implantation of a slow release or sustained release system, by suppository, or, if properly formulated, orally. Formulations for these modes of administration are well known in the art, and a summary of such methods may be found, for example, in Remington's Pharmaceutical Sciences (supra).

If treatment is to be localized, such as for the treatment of superficial tumors or skin disorders, the compound can be administered topically using standard topical compositions, such as lotions, suspensions, or pastes.

The quantity of the photosensitizer compound to be administered depends upon the choice of active ingredient, the condition to be treated, the mode of administration, the individual subject, and the judgment of the practitioner. Depending

Example 1: Biological testing

The tested compounds (see Tables 1-4 above) were dissolved in DMSO with the exception of $T(p\text{-}SO_3H)PC(OH)_2$ (123) which was dissolved in water. The solubility of the compounds was tested by placing the drug (1 mg) in 1 mL of DMSO and then spinning at 10000 rpm for 10 minutes and checking for pellet formation. The concentration of the compounds that formed a pellet was decreased from 1 mg/mL to 0.5 mg/mL DMSO and retested to show an absence of pellet.

Phototoxicity was determined with L1210 cells in the presence of the compound as follows: the L1210 cells were exposed to varying concentrations of the compounds in 96-well microtiter plates for one hour at 37°C and 5% CO_2. No fetal calf serum (FCS) was added at this time. The plate was then illuminated for one hour after which a 10% aqueous FCS solution was added to the wells. The plates were then returned to the CO_2 incubator overnight. After incubation, the cells were assayed for viability using the MTT assay (Mossman, T. J. Immunol. Meth. 1983, 65, 55).

Dark cytotoxicity was determined while the plates were wrapped in aluminum foil while under the light source.

Example 2: General chlorin synthesis

Tetraphenylporphyrin (1 g, 1.63 mmol) was dissolved in a solution of 2-10% pyridine in reagent grade chloroform. The volume of solvent used was the minimum amount required to dissolve the particular porphyrin being used and ranged from 0.25 to 1 mL/mg. Osmium tetraoxide (450 mg, 1.1 eq) was added to the solution and reaction stirred at room temperature in the dark. The reaction progress was monitored by TLC or UV-Visible spectroscopy until no further reaction was observed (3-5 days). The reaction was then purged with hydrogen sulfide gas for 10 minutes, and then purged with air until the solvent had evaporated. The solid was then dissolved in a 10% $MeOH:CHCl_3$ solution and filtered. The filtrate was evaporated to dryness and chromatographed (silica, 5 % $MeOH:CHCl_3$) to yield the analogous diol chlorin in 40-60 % yield.

$H_2TPC(OH)_2$ (compound 3) (see Bruckner, C.; Dolphin, D. Tetrahedron Lett. 1995, 36, 9425) Rf 0.7(Silica- CH_2Cl_2); UV-Vis (CH_2Cl_2) max 416, 520, 546, 594, 644 nm; ^{1}H-NMR (200 MHz, $CDCl_3$) = -1.80 (br s, 2H), 3.12 (s, 2H), 6.36 (s, 2H), 7.72-7.82 (m, 12H), 7.80 (d, 2H), 8.10 (s, 4H), 8.15 (d, 2H), 8.35 (d, 2H), 8.44 (s, 2H), 8.64 (d, 2H). MS (EI, 320°C) m/e 648 (M+, 100%).

$T(m\text{-}NO_2)PC(OH)_2$ (compound 4) Rf 0.76 (Silica-2% MeOH:$CHCl_3$); UV-Vis (CH_2Cl_2) max 412, 518, 548, 548, 594, 644 nm; ^{1}H-NMR (200 MHz, $CDCl_3$) = -1.80 (br s, 2H), 6.78 (d, 2H, Hp), 7.42 (t, 2H, Hm), 7.67 (br m, 2H, Ho), 7.86 (2d, 2H,Ho), 7.95 (m, 4H, Hm and Hp), 8.14 (d, 2H, Ho), 8.27 (br m, 2H, Ho), 8.38 (d, 2H, H), 8.48 (s, 2H, H), 8.58 (d, 2H, H); MS (EI, 320°C) m/e 828 (M+, 30%), 810 (M+ - H_2O, 100%).

$T(p\text{-}Br)PC(OH)_2$ (compound 5) Rf 0.65 (Silica- 2% MeOH:$CHCl_3$); UV-Vis (CH_2Cl_2) max 412, 486 (sh), 516, 544, 592, 648 nm; ^{1}H-NMR (200 MHz, $CDCl_3$) =-1.80 (br s, 2H), 8.0 (br s, 2H, Ho), 8.2 (br s, 6H, Ho), 8.3 (d, J = 4.76 Hz, 2H, H), 8.4 (d, J = 7.55 Hz, 8H, Hm), 8.45 (s, 2H, H), 8.60 (d, J = 4.72 Hz, H); MS (EI, 320°C) m/e 964 (M+, 10%), 946 (M+ - H_2O, 100%).

$T(m\text{-}Br)PC(OH)_2$ (compound 6) Rf 0.74 (Silica- 2% MeOH:$CHCl_3$); UV-Vis (CH_2Cl_2) max 418, 514, 548, 586, 644 nm; ^{1}H-NMR (400 MHz, $CDCl_3$) =-1.85 (br s, 2H), 6.88 (d, 2H, Hp), 7.39 (t, 2H, Hm), 7.57 (br m, 2H, Ho), 7.74 (2d, 2H, Ho), 7.83 (m, 4H, Hm and Hp), 8.04 (d, 2H, Ho), 8.07 (br m, 2H, Ho), 8.32 (d, 2H, H), 8.46 (s, 2H, H), 8.63 (d, 2H, H); MS (EI, 320°C) m/e 964 (M+, 15%), 946 (M+ - H_2O, 100%).

T(p-CO_2Me)PC(OH)$_2$ (compound 12) Rf 0.71 (Silica- 2% MeOH:$CHCl_3$); UV-Vis (CH_2Cl_2) max 422, 518, 558, 600, 646 nm; ^{1}H-NMR (200 MHz, $CDCl_3$) = -1.80 (br s, 2H), 8.0 (br s, 2H, Ho), 8.2 (br s, 6H, Ho), 8.28 (d, J = 4.76 Hz, 2H, H), 8.38 (d, J = 7.55 Hz, 8H, Hm), 8.43 (s, 2H, H), 8.59 (d, J = 4.72 Hz, H); MS (EI, 320°C) m/e 880 (M+, 65%), 862 (M+ -H_2O, 100%).

T(p-OCOEt)PC(OH)$_2$ (compound 13) Rf 0.66 (Silica- CH_2Cl_2); UV-Vis (CH_2Cl_2) max 416, 514, 548, 592, 644 nm; ^{1}H-NMR (200 MHz, $CDCl_3$) = 7.30 (br s, 2H, Ho), 7.5 (br s, 6H, Ho), 8.0 (m, 8H, Hm), 8.2 (d, 2H, H), 8.4 (s, 2H, H), 8.55 (d, H).

T(p-OMe)PC(OH)$_2$ (compound 14) Rf 0.67 (Silica-2% MeOH:$CHCl_3$); UV-Vis (CH_2Cl_2) max 420, 520, 556, 596, 648 nm; ^{1}H-NMR (400 MHz, $CDCl_3$) = -1.8 (br s, 2H), 4.1 (2s, 12H), 6.4 (s, 2H), 7.3 (m, 12H), 7.7 (d, 2H), 8.1 (m, 6H), 8.3 (d, 2H), 8.5 (s, 2H), 8.7 (d, 2H); MS (EI, 320°C) m/e 814 (ZnM+-H_2O, 15%).

T(m-OMe)PC(OH)$_2$ (compound 15) Rf 0.6 (Silica- CH_2Cl_2); UV-Vis (CH_2Cl_2) max 416, 516, 546, 584, 646 nm; ^{1}H-NMR (200 MHz, $CDCl_3$) = 4.0 (2s, 12H), 6.28 (s, 2H), 7.0 (d, 2H), 7.1 (d, 2H), 7.30 (m, 2H), 7.5 (m, 4H), 8.3 (d, 2H), 8.4 (s, 2H), 8.5 (d, 2H); MS (EI, 320°C) m/e 768 (M+, 10%), 750 (M+ - H_2O, 100%).

T(m,m'-OMe)PC(OH)$_2$ (compound 16) Rf 0.43 (Silica- CH_2Cl_2); UV-Vis (CH_2Cl_2) max 416, 520, 548, 592, 646 nm; ^{1}H-NMR (400 MHz, $CDCl_3$) = -1.85 (s, 2H), 3.9 (m, 12H), 6.4 (s, 2H), 6.8 (s, 2H), 6.85 (s, 2H), 7.1 (s, 2H), 7.25 (s, 2H), 7.3 (s, 2H), 7.4 (s, 2H), 8.4 (d, 2H), 8.6 (s, 2H), 8.75 (d, 2H).

T(o,p,o`-Me)PC(OH)$_2$ (compound 22) Rf 0.94 (Silica- 5% MeOH:$CHCl_3$): UV-Vis (CH_2Cl_2) max 418, 480 (sh), 516, 542, 592, 646 nm; ^{1}H-NMR (200 MHz, $CDCl_3$) = -1.78 (br s, 2H), 6.0 (s, 2H), 7.25 (m, 8H, Hm), 8.15 (d, 2H, H), 8.28 (s, 2H, H), 8.45 (d, 2H, H); MS (EI, 320°C) m/e 816 (M+, 30%), 798 (M+ - H_2O, 100%).

T(p-SO_3H)PC(OH)$_2$ (compound 23) Rf 0.1 (Silica- CH_2Cl_2); UV-Vis (CH_2Cl_2) max 420, 520, 548, 592, 648 nm.

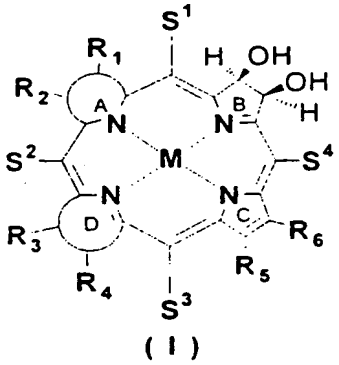
T(p-t-Bu)PC(OH)$_2$ (compound 24) Rf 0.75 (Silica- CH_2Cl_2); UV-Vis (CH_2Cl_2) max 418, 522, 548, 594, 644 nm; ^{1}H-NMR (200 MHz, $CDCl_3$) = 3.45 (2s, 36H, Me), 6.2 (s, 2H), 7.0 (m, 8H, Hm), 7.5 (m, 4H, Ho), 7.8 (m, 2H, Ho), 8.0 (br m, 2H, Ho), 8.3 (d, 2H, H), 8.4 (s, 2H, H), 8.6 (d, 2H, H); MS (EI, 320°C) m/e 935 (M+, 10%), 916 (M+ - H_2O, 60%).

H_2DPC(OH)$_2$ (compound 25) Rf 0.3 (Silica- CH_2Cl_2); UV-Vis (CH_2Cl_2) max 402, 504, 530, 584, 638 nm; ^{1}H-NMR (200 MHz, $CDCl_3$) = -2.1 (br s, 1H), -1.9 (br s, 1H), 6.05 (d, J= 6.3 Hz, 1H), 6.35 (d, J= 6.4 Hz, 1H), 7.65 (m, 6H), 7.9 (d, 1H), 8.10 (d, J= 4.4 Hz, 2H), 8.25 (d, 1H), 8.45 (d, J= 4.5 Hz, 1H), 8.65 (d, J= 4.5 Hz, 1H), 8.80 (d, J= 4.5Hz, 1H), 8.95 (d, J= 4.5 Hz, 1H), 9.0 (d, J=4.4 Hz, 1H), 9.15 (d, J= 4.3 Hz, 1H), 9.90 (s, 1H), 9.34 (s, 1H); MS (EI) m/e calc'd for $C_{32}H_{24}N_4O_2Ni$: 496.18945, found 496.18923 (M+, 100%).

mono p-Br PC(OH)$_2$ (compound 26) Rf 0.4 (Silica- CH_2Cl_2); UV-Vis (CH_2Cl_2) max 412, 508, 528, 596, 644 nm; ^{1}H-NMR (200 MHz.$CDCl_3$) = 6.2 (d, 2H), 7.0 (d, 2H), 7.5 (m, 12H), 7.8 (2d, 1H), 8.0 (m, 4H), 8.2 (d, 1H), 8.3 (d, 1H), 8.35 (s, 2H), 8.5 (d, 1H); MS (EI, 320°C) m/e 726 (M+, 20%), 709 (M+ - H_2O, 100%).

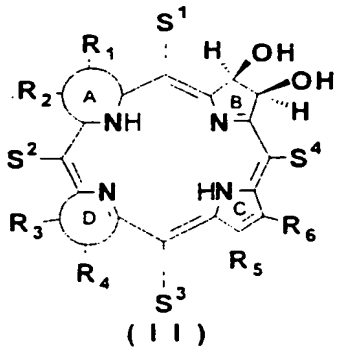
We claim:

1. A pharmaceutical composition comprising an improved β,β'-dihydroxy meso-substituted chlorin. bacteriochlorin or isobacteriochlorin compound having the formula (I) or (II):



(I)

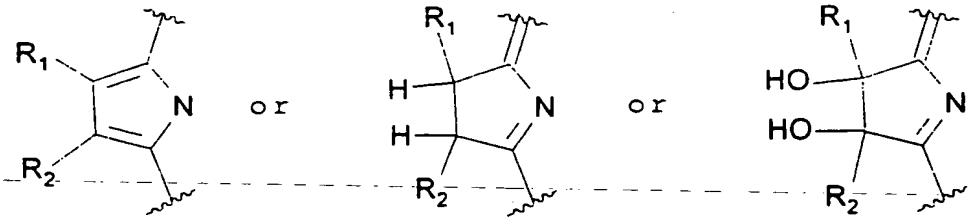
or



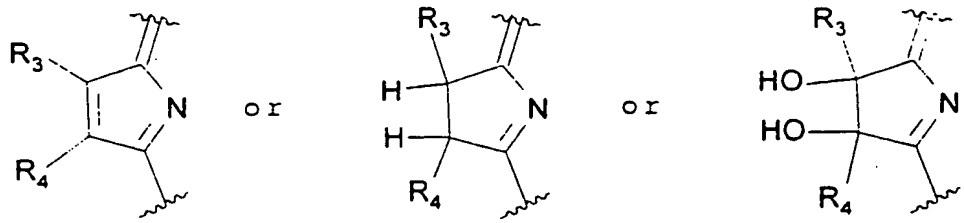
(II)

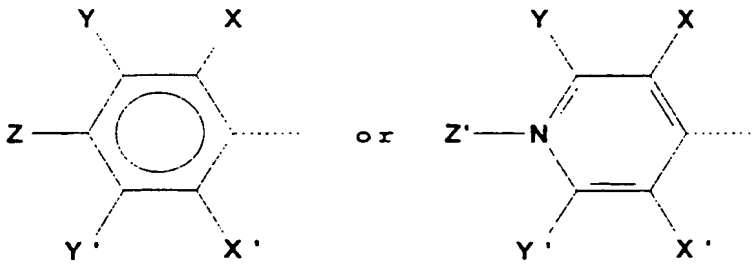
wherein M is a metal selected from the group consisting of Ni(II), Cu(II), Zn, Sn, Ge, Si, Ga, Al, Mn(III), Gd(III), In and Tc;

A is a ring having the structure:



D is a ring having the structure:





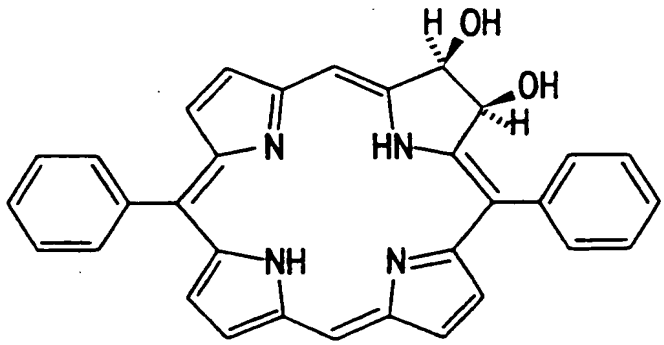
wherein X, X', Y, Y' and Z are independently hydrogen, halogen, lower alkyl, lower alkoxy, hydroxy, carboxylic acid or acid salt, carboxylic acid ester, sulfonic acid or acid salt, sulfonic acid ester, substituted or unsubstituted amino, cyano, nitro, or a biologically active group, and Z' is hydrogen or lower alkyl.

8. The composition of claim 7 wherein X, X', Y, Y' and Z are selected from the group consisting of hydrogen, methyl, ethyl, t-butyl, methoxy, hydroxy, OR where R is an alkyl group or a fatty acid group having from 6 to 18 carbon atoms, fluoro, chloro, iodo, bromo, $-C(O)-OCH_3$, cyano, nitro, or a ligand specific for a biological receptor.

9. The composition of claim 7 wherein X, X', Y and Y' are each hydrogen, and Z is selected from the group consisting of hydrogen, halogen, lower alkyl, lower alkoxy, hydroxy, carboxylic acid or acid salt, carboxylic acid ester, sulfonic acid ester, sulfonic acid or acid salt, nitro, amino, cyano, and a biologically active group.

10. The composition of claim 7 wherein at least one of X, X', Y, Y' and Z is a biologically active group or a substituent that increases the amphiphilic nature of the molecule.

11. The composition of claim 1 wherein said improved compound is selected from the group consisting of compounds 3 to 30.



(125)

FIG. 1

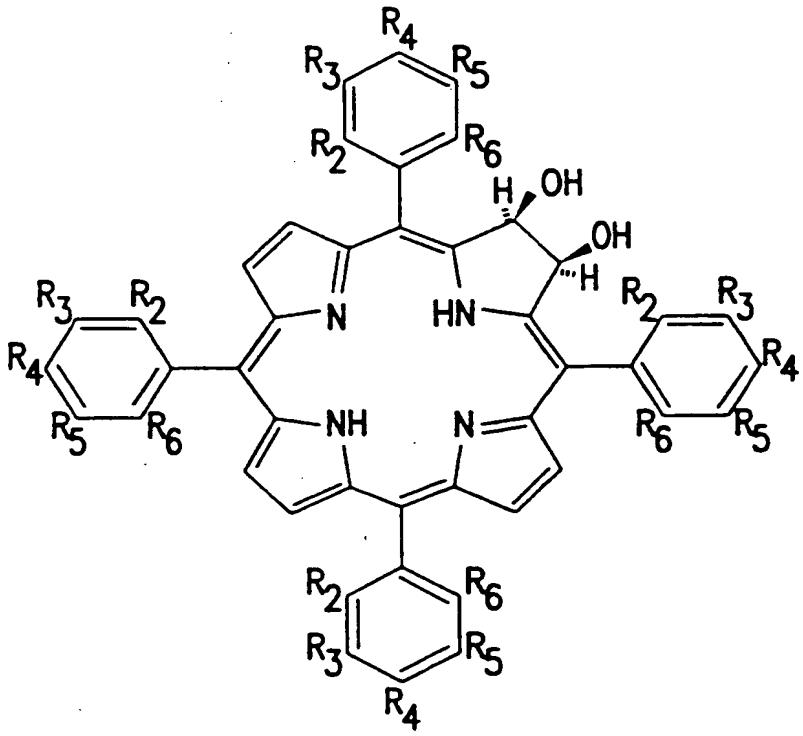
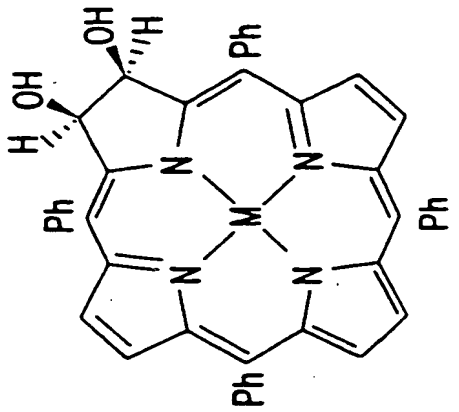
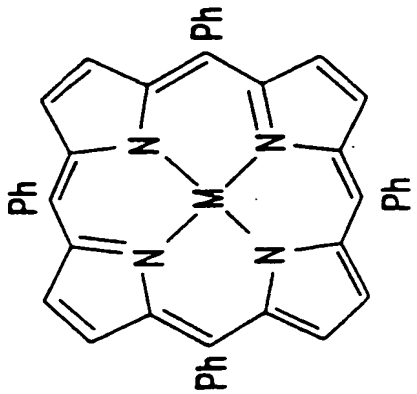


FIG.3



Tetraphenyldihydroxychlorin
M = H_2, Zn, Ni
(64)

1. 1eq. OsO_4
Py/$CHCl_3$
2. H_2S



Tetraphenylporphyrin
M = H_2, Zn, Ni
(10)

FIG.5

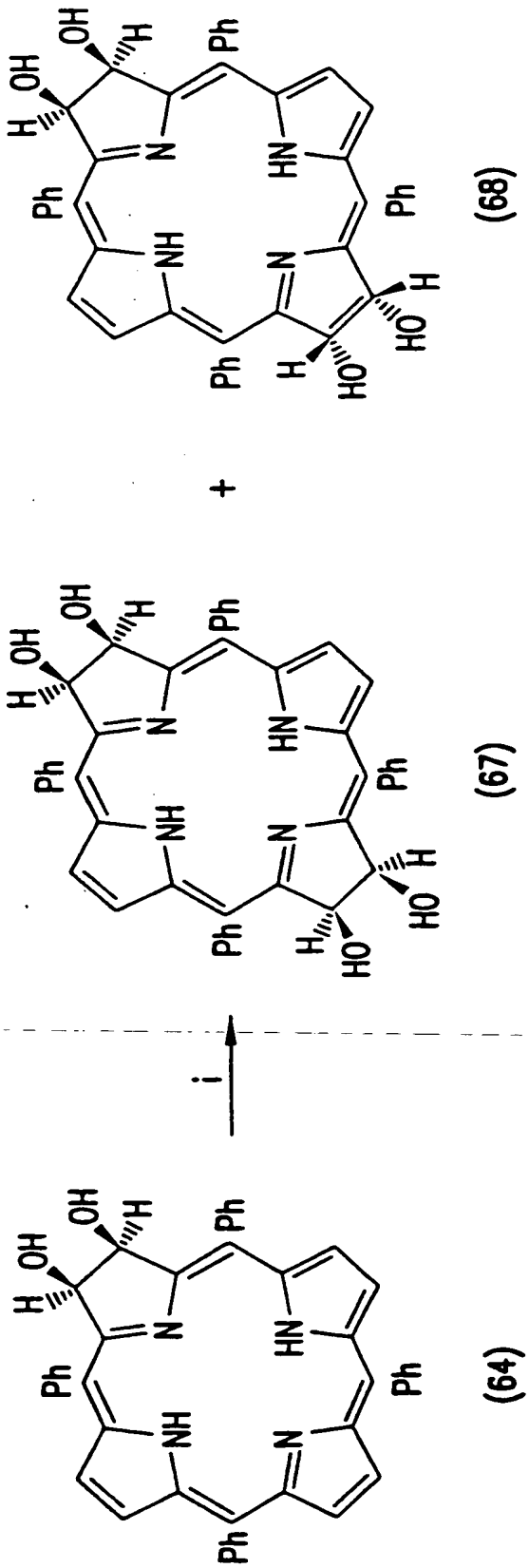


FIG.7

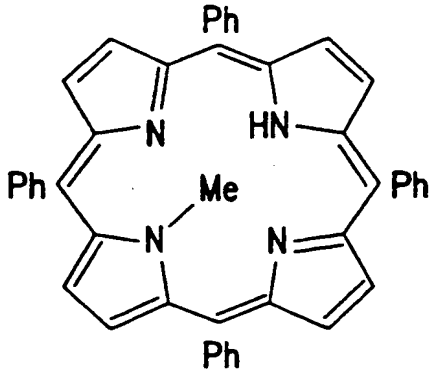


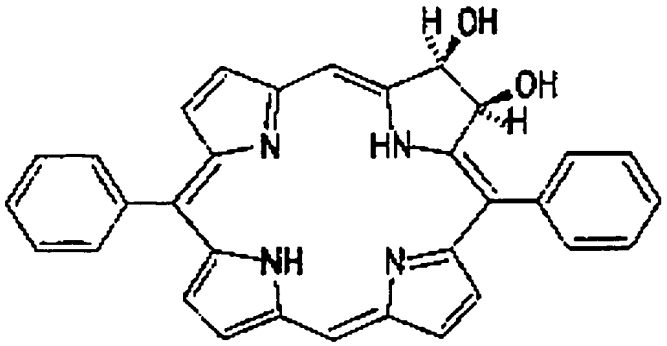
FIG.9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 00/00435

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9613504 A	09-05-1996	US 5648485 A	15-07-1997
		AU 704971 B	13-05-1999
		AU 3695195 A	23-05-1996
		CA 2199399 A	09-05-1996
		CN 1161697 A,B	08-10-1997
		CZ 9701155 A	17-09-1997
		EP 0804439 A	05-11-1997
		FI 971734 A	23-04-1997
		HU 77008 A	02-03-1998
		JP 10507766 T	28-07-1998
		NO 971952 A	25-04-1997
		NZ 294203 A	23-12-1998
		PL 319907 A	01-09-1997
		US 5831088 A	03-11-1998



(125)

FIG. 1

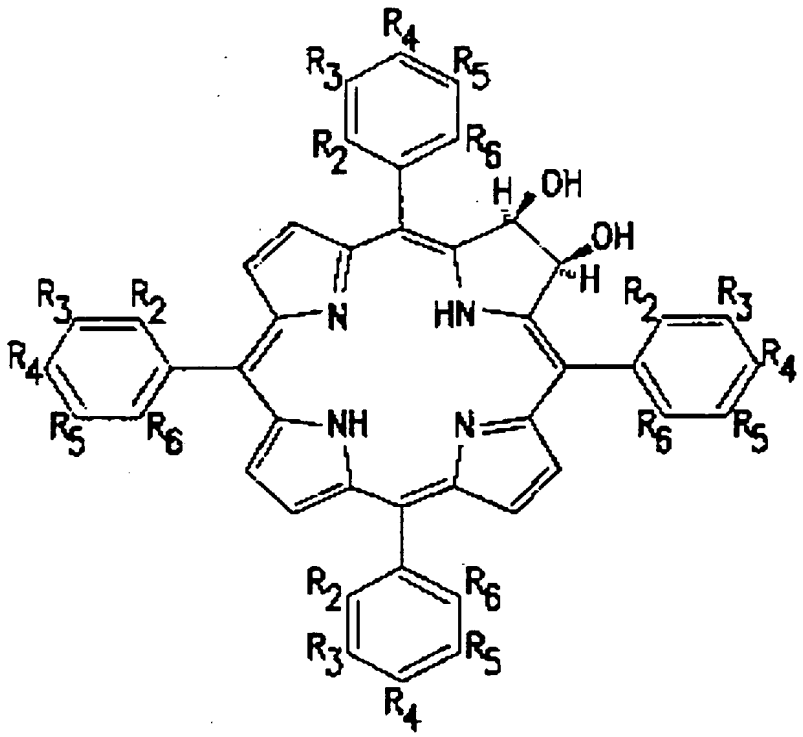
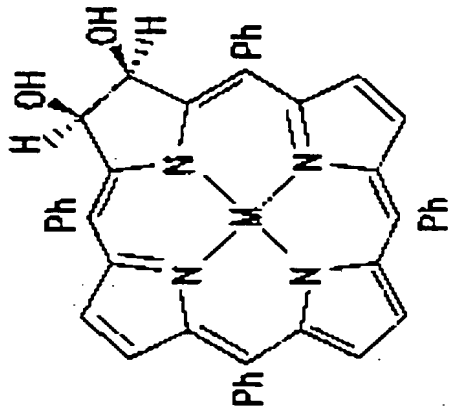
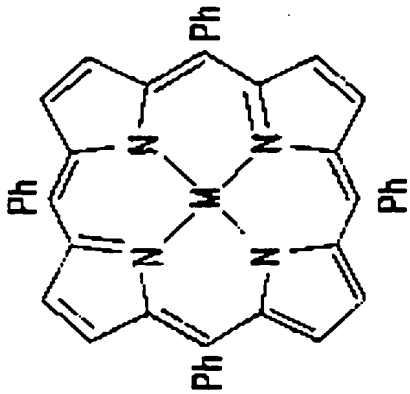


FIG.3



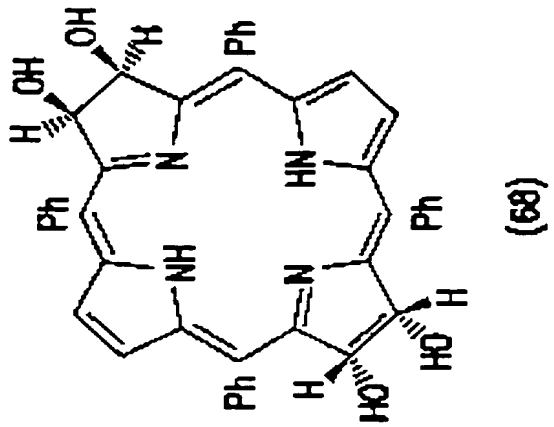
Tetraphenyldihydroxychlorin
M = H_2, Zn, Ni
(64)

1. 1eq. OsO_4
Py/$CHCl_3$
2. H_2S



Tetraphenylporphyrin
M = H_2, Zn, Ni
(10)

FIG.5



(68)

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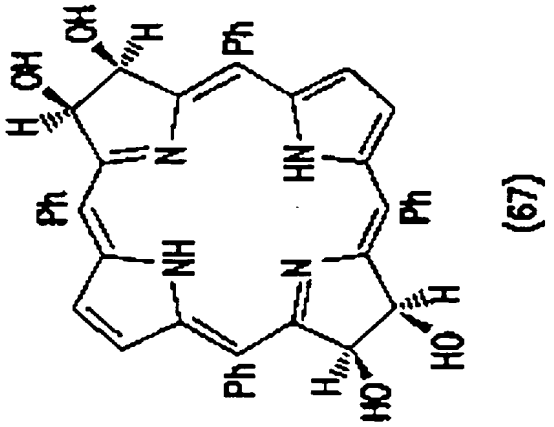
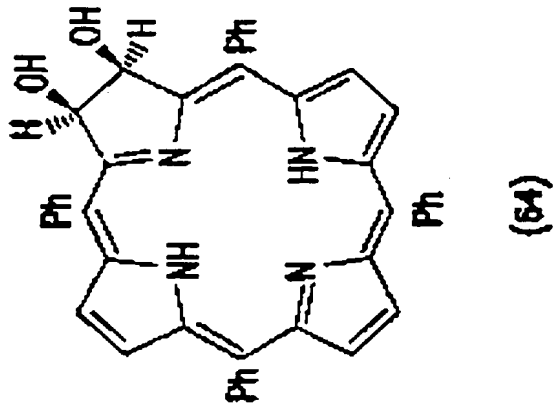


(67)

FIG.7



(64)

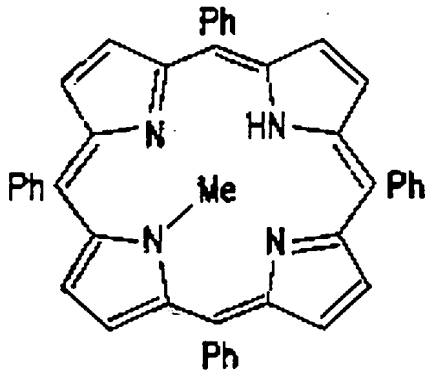


FIG.9